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Title: Biogeochemical Cycling and Environmental Stability of Pu Relevant to Long-Term Stewardship of DOE Sites

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Results To Date: Biogeochemical Cycling and Environmental Stability of Pu Relevant to Long-Term Stewardship of DOE Sites(June 2006)

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I. Overview. The overall objective of this proposed research is to understand the biogeochemical cycling of Pu in environments of interest to long-term DOE stewardship issues. Central to Pu cycling (transport initiation to immobilization) is the role of microorganisms. The hypothesis underlying this proposal is that microbial activity is the causative agent in initiating the mobilization of Pu in near-surface environments: through the transformation of Pu associated with solid phases, production of extracellular polymeric substances (EPS) carrier phases, and the creation of microenvironments. Also, microbial processes are central to the immobilization of Pu species, through the metabolism of organically complexed Pu species and Pu associated with extracellular carrier phases and the creation of environments favorable for Pu transport retardation.

This project is collaboration among the Colorado School of Mines (Honeyman, Diaz and Tinnacher), Brookhaven National Laboratory (Francis, Gillow and Dodge) and Texas A&M University (Santschi, Hung, Schwehr, Roberts). This report outlines the results of work performed by this collaborative group during the second reporting phase of this project (approximately June 1 2005 - June 15, 2006).

Because the RIMS system does not support special characters, figures, equations or tables, the full report is available from the NABIR program managers or the PI (honeyman@mines.edu). The text below is an extended summary of the project results to date.

The sub-projects focused on this year include:
A. Characterization of Pu Contaminated Soil by XRF
B. Biotransformation of Pu in Contaminated Soil
C. Production, Isolation and characterization of exopolymeric substances (EPS).
D. Production, isolation and characterization of Pu-organic iron-containing colloids from RFET soils that have been shown to be one of the vectors for dispersal of Pu.
E. Immobilization potential of bacterial EPS for Pu bound to different colloidal phases.
F. 'Static' Column Experiments

G. Pu binding to EPS isolates.

II. Details of Individual Sub-Projects.

Summary of Characterization of Contaminated Soil and Batch Microcosm Studies.

1. Soil collected from RFETS was analyzed by synchrotron x-ray absorption spectroscopy at the new microprobe beamline at NSLS (X27A). Plutonium was below the detection limit, however elemental analysis by micro-x-ray fluorescence ((SXRF) showed that soils collected in 1997 and 2004 were very similar in composition. Soil microparticulates and colloids (<2 micron) were composed of soil mineral fragments and iron was a major constituent. The iron was a principal sorptive phase for Pu as shown by (SXRF mapping upon addition of 242Pu spike to the <2 micron fraction. The iron content of the soil ranged from 0.02 to 0.04 wt. % poorly crystalline iron oxide and 0.5 wt. % reducible iron oxide.

2. The addition of electron donors glucose and lactate in batch microcosm experiments with the RFETS soil resulted in the stimulation of soil microorganisms with concomitant production of organic acid metabolites, total gas, and a decrease in pH and Eh. In the microcosms with 1997 soil, 2.4-4.1 % of the total ambient 239,240Pu concentration (1.1 x 10-9 M) was mobilized (in the unfiltered fraction) due to microbial activity at 45 days incubation. Similarly, microcosms with 2004 soil resulted in 0.4% of the ambient 239,240Pu-detected in the <0.45micron fraction of glucose incubated samples (>2x the concentration of 239.240Pu detected in the unamended control samples).

3. The addition of 242Pu as a low-activity, high concentration tracer for Pu to soil samples collected in 1997 or 2004, amended with glucose, and incubated for 54 days and 77 days, respectively showed remobilization of 0.4 % of the total concentration of added 242Pu in the colloidal fraction. However, the majority of the ambient and added Pu remained immobilized and associated with particles >0.45 (m.

C. Production, Isolation and characterization of exopolymeric substances (EPS). EPS were isolated by the TAMUG group from three bacterial species: a) two aerobic soil bacteria: Shewanella putrefaciens CN32, and Pseudomonas fluorescens Biovar II; and b) one anaerobic bacterium, Clostridium sp. BC1.

Purity test of EPS: The results shown in Figure 11a suggest that EPS purity was very high after four times ethanol precipitation, with 90% of the C-14 tracer recovered. [Figure 11a and b: available with the full report.].

The neutral monosaccharide and acidic functional group composition in EPS from Pseudomonas fluorescens Biovar II was reported in previous year's progress report. More progress has been made this year with analyses of the protein content, which renders the EPS amphiphatic (amphiphilic), as shown in Figure 12.

Figure 12.

D. Production, isolation and characterization of Pu-organic iron-containing colloids from RFETS soils that have been shown to be one of the vectors for dispersal of Pu. Colloidal Pu was extracted from resuspending 1 kg of Pu-contaminated soils from Rocky Flats Environmental Technology Site (RFETS) into distilled water by the TAMUG group. This allows us to separate Pu-binding macromolecules by gel electrophoresis and isoelectric focusing (IEF, 2-dimensional polyacrylamide gel

electrophoresis, 2-D PAGE) (Santschi et al., 2002a,b; Alvarado-Quiroz et al., 2005). These colloids were then characterized in terms of %C, %N, and Pu activity concentration. Results of these analyses are shown in Table 3, where they are compared to the composition of the original soil.

[Table 3.]

While these colloids are enriched in organic carbon by an order of magnitude, they are also enriched for Pu, when compared to soil, in which Pu concentrations in RFETS soil are inversely dependent to size (Figure 13).

[Figure 13.]

These results confirm previous results of stream colloids and stream particulates from the RFETS site (Santschi et al., 2002), whereby colloids were also enriched in both organic carbon and in Pu. Last, but not least, several 100 mg of colloids had been made available for X-ray Spectroscopic Techniques (XANES, EXAFS) and other spectroscopic quantifications by the BNL group, as well as mobility and titration experiments by the CSM group.

Pu and organic carbon content of RFETS soil samples, determined by the TAMUG group using ion chromatography, the concentration of anions (chloride, nitrite, nitrate, phosphate, and sulfate), for water leachates from Rocky Flats soil fractions and bulk samples were reported in last years progress report. Pu in the RFETS colloids was added to an Isoelectric Focusing System, together with Th(IV) tracer added, and the resulting spectrum is shown in Figure 14.

[Figure 14.]

The pH of 3 sections was subsequently extracted from the gel, purified using ultrafiltration, freeze-dried and processed for carbohydrate analysis. The resulting relative monosaccharide composition of the pH 3 section is shown in Figure 15.

[Figure 15.]

E. Immobilization potential of bacterial EPS for Pu bound to different colloidal phases. Microbially produced EPS can act as a sorptive sponge or colloid trap (Hoffman and Decho, 1999) through a combination of steric, hydrophobic and hydrophilic interactions. Confirmation of such a colloid trap model come from the AFM and TEM results (Santschi et al., 1998, Wilkinson et al., 1999), which show abundant fibrils covered by smaller spherical colloids. Colloid trapping by mineral particles appears to be responsible also for the observed enhancement of the Kd value for Pu(IV) onto mineral particles such as silica, as compared to the linear sum of the individual Kd values (e.g., calculated from silica and EPS as end-members). Experiments with high-purity chemicals, 'cleaned-up' chemicals and solutions with Chelex and XAD resins, followed by ultrafiltration, and experiments carried out in a clean-room environment were reported by the TAMUG group in last year's progress report. These experiments had shown that solutions with different clean-up histories contain colloidal impurities with different chemical and physical properties that can control the extent of sorption of Pu(IV) to particles and/or surfaces of the experimental set-up during sorption experiments.

Bacteria exude extracellular polymeric substances (EPS) that are acid polysaccharide-

rich and expected to affect the mobility and adsorption of actinides in surface and ground water. EPS are predicted to enhance adsorption onto particles, especially if they are amphiphatic and contain hydrophobic moieties, such as proteins. The results, summarized in Table 4, show that 1) EPS with protein shows higher Kd for Pu(IV) than Pu(V), when EPS was in water for < 1 day (likely less alteration); 2) Kd for Pu(IV) is 1 order of magnitude higher than for Pu(V) under identical conditions, whereby EPS was in water for 4 days.

[Table 4.]

H1. Relative hydrophobicity of biopolymers using chromatographic and computational techniques: The hydrophobicity of EPS is an important parameter, allowing one to predict the inter-polymer affinity and "stickiness". We hypothesize that Pu binding is controlled by clustered functional groups within the hydrophilic polysaccharide moiety of the macromolecular chelate, while particle surface affinity of the EPS polymeric ligand is controlled, to a large extent, by the hydrophobic parts (e.g., proteins) of the amphiphilic or amphiphatic bio-emulsifying polymer (e.g., Ron et al., 2001). Relative hydrophobicity of a polymer can be measured through octanol-water partitioning coefficient values (Kow), hydrophobic interaction chromatography (HIC) using 2 butyl 1 ml HiTrap columns in series from Amersham GE. Results on colloidal characterization by the TAMUG group involving the hydrophobic or hydrophilic interactions of EPS and APS of experimental determination of octanol-water partitioning coefficient values (Kow) via shake-flask and slow-stir methods, have been reported in last years progress report.

Experiments with EPS varieties that include amphiphatic (amphiphilic) protein-rich and hydrophilic, protein-poor EPS from capsular or solution phases, in order to vary the relative hydrophobicity of the EPS, for selection of the Pu binding ligands of different sorption strengths or attachment potential (sticky factor; Quigley et al., 2001) were carried out. In order to investigate the question of relative hydrophobicity, we produced protein-poor varieties of EPS through pronase treatment subsequent to their separation by repeated alcohol precipitation. Using a Waters HPLC system, Size Exclusion Chromatography (SEC) columns and refractive index detection, we were able to show that while the molecular weight distribution is almost identical for EPS from Pseudomonas fluorescens Biovar II with and without proteins, i.e., showing peaks at 6 and 20 kDa (Figure 16 and 17), the Hydrophobic Contact Area (HCA) determination by hydrophobic interaction chromatography-HPLC and refractive index detection gave very different results (Figure 18). It was found that the HCA of protein-containing EPS from Pseudomonas fluorescens Biovar II is considerably higher than that of the protein-poor variety, i.e., 12.5 vs. 1.8 Angstroms^2 molecule-1 (Figure 18).

F. 'Static' Column Experiments

Various static columns have been conducted employing Rocky Flats (RF) soil and the stimulation of indigenous soil microorganisms by the addition of glucose as an electron donor. Static columns allow for batch incubation conditions as well as the investigation of plutonium (Pu) mobilization. Static column experiments were compared to batch systems, where 0.33% of the 239,240Pu present in the contaminated soil was solubilized. According to our results, no enhanced mobilization of 239,240Pu occurred despite the presence of microbial activity in the glucose-stimulated columns. Microbial activity was confirmed by detecting low molecular weight organic acids (i.e., fermentation metabolites) in the column effluent.

In addition to incubation experiments, static columns were used to investigate the

behavior of tracer 241Pu in the presence of dissolved Pseudomonas fluorescens exocellular polymeric substance (EPS); a non-aqueous phase colloidal organic ligand. 241Pu was sorbed to the RF soil in the static columns. An initial flushing with a control solution or 22mg EPS/L was conducted. Another flushing was conducted after 24 hours of solution contact with the RF soil. The data for both flushing events are presented in Figure 20.

A clear enhancement in 241Pu mobilization occurs in the presence of EPS. This is an important result considering the fact that microbial activity is present in virtually every environment and therefore so is EPS. In Pu contaminated environments there is the potential for enhanced mobilization as a result of microbial EPS.

G. Pu binding to EPS isolates. Analysis of Pu binding to EPS isolates was done using a combination of potentiometric titrations, fitting titration data to a discrete ligand model and inverse simulation of Pu binding experiments using resin competition.

Figure 21 shows a classical pH/potentiometric titration of an alginic acid produced by soil bacteria. The alginic acid is used as a surrogate for the more difficult to produce bacterial EPS. The titration curves are interpreted using the discrete ligand approach are transformed as shown in Figure 22. The pKa values of 2, 4, 6 and 8 are arbitrarily set with the computational algorithm returning the ligand 'concentrations as shown.

Figure 23 is an example of experimental data for Pu binding to the alginic acid. The partitioning is determined using a ligand competition method (Kantar and Honeyman, 2005). The partitioning data is fit through inverse modeling using the alginic acid ligands and postulated Pu / ligand formation reactions (Table 5). In this case, Pu is postulated as binding to the pKa = 2 and 3 alginic acid ligands.

Figure 24 is a comparison of the discrete ligands composing different bacterial EPS isolates. The ligand concentrations were determined through inverse modeling of titration curves using the discrete ligand/ affinity distribution approach. Table 6 presents a comparison of the postulated Pu / EPS ligand formation reactions and stability constants derived though inverse modeling of resin exchange data.

Deliverables: Hung, C.-C., Santschi, P.H., and Gillow, J.B. 2005. Isolation and characterization of extracellular polysaccharides produced by Pseudomonas fluorescens Biovar II. Carbohydrate Polymers, 61, 141-147.

Kantar, C. and B.D. Honeyman (2005). Binding of Pu to citric and alginic acids. Radiochim. Acta., 93, 757 - 766.